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Note

Hydrophobic Components in Delipidated Granule of Egg Yolk

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Previously we reported on the isolation of low density lipoprotein (LDL) from hen's egg yolk plasma by affinity chromatography.[1] The granule, another constituent of egg yolk, was also adsorbed on lauryl-sepharose 4B (C 12-sepharose 4B). To investigate the hydrophobic properties of egg yolk granule, we examined the hydrophobic components of delipidated granule. In the present paper, we describe the chemical composition, amino acid composition and molecular weight of soluble delipidated granule which was almost adsorbed on C 12-sepharose 4B.

C 12-sepharose 4B was prepared by the cyanogen bromide method.[2] Hexose was determined by a phenol-sulfuric acid reaction as described by Dubois *et al.*[3] Hexosamine was determined by the method of Cessi and Pilliego[4] using glucosamine as a standard. Sialic acid was determined by the thiobarbituric acid assay of Warren.[5] Phosphate was determined by the method of Gomori.[6] SDS-polyacrylamide gel electrophoresis was performed using the procedure of Hayashi and Ooba[7] with 10% polyacrylamide gel at a current of 8 mA per tube. The gel was stained for protein with 0.5% amido black 10B in 20% acetic acid. The destained gel was estimated at an absorbance of 590 nm using an Atago Densitomaster Kemic. Gel filtration was performed using Sephadex G-75 with the gel packed into a glass column (2.6 × 70 cm). The sample was applied on the column and eluted with 5% sodium chloride in 0.01 M Tris-HCl, pH 8.0 and 5 ml fractions were collected. The eluate was monitored at 280 nm.

Egg yolk was diluted with an equal weight of 0.16 M NaCl, stirred and centrifuged at 4°C and 45,000 *g* for 30 min. The precipitated fraction was homogenized with 10 volumes of 0.16 M NaCl for 24 hr and centrifuged at 4°C and 45,000 *g* for 30 min. This procedure was repeated twice and the final precipitate was called granule. The granule was delipidated with 20 volumes of chloroform–methanol (2:1) and this procedure repeated twice as previously reported.[8] The delipidated residue was called whole delipidated granule. The percentage of 5% NaCl soluble delipidated granule (soluble delipidated granule) was 7.0% of the whole delipidated granule. A typical elution pattern for the soluble delipidated granule by affinity chromatography is shown in Fig. 1. Most of the soluble delipidated granule was adsorbed on C 12-

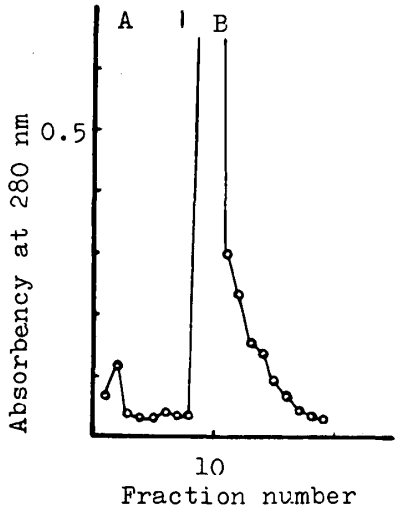

FIG. 1. Elution Profiles of Soluble Delipidated Granule on C 12-Sepharose.

The column dimensions were 0.8 cm × 10 cm and 5 ml fractions were collected. A sample (10 mg) was applied to the column, which was equilibrated with 5% sodium chloride–0.01 M Tris HCl, pH 8.0 (A), and washed with 0.5% sodium deoxycholate solution in 0.01 M Tris-HCl, pH 8.0 (B).

TABLE I. AMINO ACID COMPOSITION OF WHOLE DELIPIDATED GRANULE AND SOLUBLE DELIPIDATED GRANULE

	Whole delipidated granule μmol/ml	Soluble delipidated granule μmol/ml
Asp	4.1	3.1
Thr	2.1	0.7
Ser	4.3	16.0
Glu	5.7	2.2
Pro	2.2	0.5
Gly	1.3	0.7
Ala	2.5	1.0
½Cys	0.6	0.3
Val	3.1	0.7
Met	1.6	0.4
Ile	2.8	0.5
Leu	4.3	0.8
Tyr	2.0	0.7
Phe	1.9	1.0
Lys	3.5	4.0
His	1.3	2.6
Arg	4.2	3.4

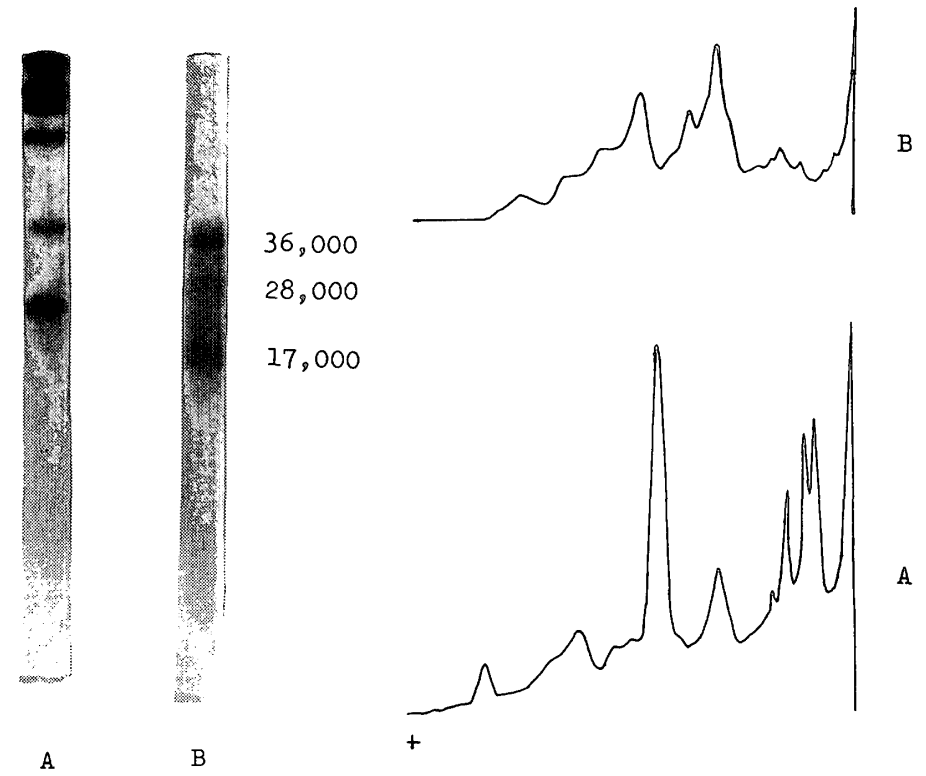

FIG. 2. SDS Polyacrylamide Gel Electrophoresis of Whole Delipidated Granule and Soluble Delipidated Granule Stained with Amido Black 10 B.

A, whole delipidated granule; B, soluble delipidated granule.

sepharose 4B and eluted with a 10 mM Tris-HCl buffer (pH 8.0) containing 0.5% sodium deoxycholate. Whole delipidated granule contained 1.91% of hexose, 0.45% of hexosamine and 0.21% of sialic acid. On the other hand, soluble delipidated granule contained 1.83% of hexose, 0.51% of hexosamine and 0.74% of sialic acid. The phosphate content of whole delipidated granule and soluble delipidated granule was 0.9% and 7.2%, respectively, so soluble delipidated granule contained more sialic acid and phosphate than whole delipidated granule. The amino acid composition of whole delipidated granule and soluble delipidated granule is shown in Table I. Soluble delipidated granule contained 41.5% of serine and showed a different amino acid composition in contrast to whole delipidated granule. According to Powrie,[9] granule comprises 70% of high denisity lipoprotein (lipovitellin), 12% of LDL and 16% of phosvitin. Evans *et al.*[10] also isolated lipovitellin from egg yolk granule and analyzed the amino acid composition of lipovitellin. According to their report, lipovitellin contained 5.7% of serine. Kurisaki *et al.*[11] isolated lipovitellin into two components, namely α-lipovitellin and β-lipovitellin and they reported that α-lipovitellin contained 8.32% of serine and 0.39% of phosphate and β-lipovitellin contained 7.59% of serine and 0.19% of phosphate. But our soluble delipidated granule contained 41.5% of serine and 7.2% of phosphorus. So it seems that this fraction contained proteins other than apolipovitellin (vitellin) and we assume that the main fraction was phosvitin. Allerton and Perlman[12] analyzed the amino acid composition of phosvitin and they reported that phosvitin contained 30.0% of serine and no cystine. On the other hand, Clark[13,14] reported that the serine content of phosvitin was 54.0% and Ho *et al.*[15] reported that the phosphate content of phosvitin was 10.0%. In this way, phosvitin contained more serine and phosphate. So it seems that our soluble delipidated granule contained a large amount of phosvitin. SDS-polyacrylamide gel electrophoretic patterns of whole delipidated granule and soluble delipidated granule are shown in Fig. 2. Whole delipidated granule consisted of at least seven polypeptide chains and their molecular weights were 130,000, 92,000, 84,000, 71,000, 39,000, 22,000 and 10,000. The molecular weights of the main component of whole delipidated granule were 130,000, 92,000, 84,000, 71,000 and 22,000. Hilliard *et al.*[16] have reported that the molecular weight of the main component of vitellin was 30,100 and that for the minor components was 20,000 and 24,000, whilst Franzen and Lee[17] delipidated lipovitellin and analyzed the molecular weights of vitellin as 30,000 and 44,000. But the molecular weights of the soluble delipidated granule components were 36,000, 28,000 and 17,000. Clark[13] isolated phosvitin from egg yolk and reported that the molecular weight of the major fraction was 34,000 and that of the minor fraction was 28,000. So these values almost agreed with our value for main soluble delipidated granule. However, the component which had a molecular weight of 17,000 does not correspond with any of the polypeptides which have been reported up to now. As we expected, the

main component of soluble delipidated granule was phosvitin. Phosvitin contains more phosphate radicals and it has been reported that phosvitin combines with some kind of cationes.[18~21] But according to the hydrophobic properties of phosvitin, it has been reported that phosvitin only makes a complex with lipovitellin,[22] so it is new finding that phosvitin makes a hydrophobic bond with C 12-Sepharose 4B.

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